

[Application No. 08/300,484]

unprotected 3'-hydroxyl group of said initiating substrate and a 5'-phosphate of said nucleoside 5'-triphosphate, so as to add said nucleoside to said initiating substrate.

²~~40~~. The method of claim ¹~~39~~, wherein said enzyme is a template-independent polynucleotide polymerase.

³~~41~~. A method as in claim ¹~~39~~ or ²~~40~~ further comprising:

c) removing the blocking moiety protecting the 3' position of said nucleoside 5'-triphosphate to produce an initiating substrate having an unprotected 3'-hydroxyl group.

⁴~~42~~. The method of claim ³~~41~~ further comprising repeating steps (b) and (c) at least once.

⁵~~43~~. The method of claim ³~~41~~ further comprising repeating the steps (b) and (c) until the polynucleotide having the predetermined sequence is obtained.

⁶~~44~~. A method as in claim ¹~~39~~ or ²~~40~~, wherein said initiating substrate is selected from the group consisting of ribonucleosides, deoxynucleosides, nucleotides, and single and double stranded oligonucleotides and polynucleotides.

⁷~~45~~. A method as in claim ¹~~39~~ or ²~~40~~, wherein said initiating substrate further comprises oligonucleotide sequences.

⁸~~46~~. The method of claim ⁷~~45~~, wherein said oligonucleotide sequences are attached to non-nucleoside molecules.

⁹~~47~~. A method as in claim ¹~~39~~ or ²~~40~~, wherein said initiating substrate is immobilized on a solid support.

¹⁰~~48~~. The method of claim ⁹~~47~~, wherein said solid support is selected from the group consisting of cellulose, controlled-pore glass, silica, polystyrene, styrene divinyl benzene, agarose and crosslinked agarose.

¹¹~~49~~. The method of claim ²~~40~~, wherein said template-independent polynucleotide polymerase is terminal deoxynucleotidyl transferase.

¹²~~50~~. A method as in claim ¹~~39~~ or ²~~40~~, wherein said removable blocking moiety is removed in under 10 minutes to produce a hydroxyl group at the 3' position of the 3'-terminal nucleoside.

¹³~~51~~. The method of claim ¹²~~50~~, wherein said removable blocking moiety is removed in under 2 minutes to produce a hydroxyl group at the 3' position of the 3'-terminal nucleoside.

¹⁴~~52~~. A method as in claim ¹~~39~~ or ²~~40~~, wherein said removable blocking moiety is selected from the group consisting of esters, ethers, carbonitriles, phosphates, phosphoramidate, carbonates, carbamates, borates, nitrates, sugars, phosphoramidates, phenylsulfenates, sulfates, and sulfones, wherein said removable blocking moiety is linked to the 3' carbon of said nucleoside 5'-triphosphate.

[-2-]

D¹
cont

65

[Application No. 08/300,484]

¹⁵₅₃. A method as in claim ¹₃₉ or ²₄₀, wherein said removable blocking moiety is selected from the group consisting of an ester, a phosphorous containing moiety and an ether.

¹⁶₅₄. The method of claim ¹⁵₅₃, wherein said ester is selected from the group consisting of toluoyl ester, isovaleroyl ester, benzoyl ester, 4-nitrobenzoyl ester, 2,6 dimethylbenzoyl ester, 3,5 dimethylbenzoyl ester and dimethylbenzoyl ester.

¹⁷₅₅. The method of claim ¹⁵₅₃, wherein said ether is selected from the group consisting of bis(2-chloroethoxy)methyl ether, 4-methoxytetrahydropyranyl ether, tetrahydrofuranlyl ether, 1-ethoxyethyl ether, tri(p-methoxyphenyl)methyl ether, di(p-methoxy)phenylmethyl ether, t-butyldimethylsilyl ether.

¹⁸₅₆. The method of claim ¹⁵₅₃, wherein said phosphorous containing moiety is selected from the group consisting of phosphate, phosphoramidate and phosphoramidate.

¹⁹₅₇. A method as in claim ¹₃₉ or ²₄₀, further comprising treating said nucleoside 5'-triphosphate having said removable blocking moiety with a deblocking solution whereby said removable blocking moiety is removed.

²⁰₅₈. The method of claim ¹⁹₅₇, wherein said deblocking solution comprises a divalent cation.

²¹₅₉. The method of claim ²⁰₅₈, wherein said divalent cation is Co⁺⁺.

²²₆₀. The method of claim ¹⁹₅₇, wherein said deblocking solution comprises a buffer selected from the group consisting of dimethylarsinic acid, tris[hydroxymethyl] amino methane and 3-[m-morpholine] propionanesulphonic acid.

²³₆₁. The method of claim ¹⁹₅₇, wherein said deblocking solution comprises an enzyme that catalyzes the removal of said removable blocking moiety.

²⁴₆₂. The method of claim ¹⁹₅₇, wherein said treating occurs in under 10 minutes.

²⁵₆₃. The method of claim ²⁴₆₂, wherein said treating occurs in under 2 minutes.

²⁶₆₄. A method as in claim ¹₃₉ or ²₄₀, wherein said removable blocking moiety is linked to a solid support.

²⁷₆₅. The method of claim ²⁶₆₄, further comprising cleaving said polynucleotide from said solid support.

²⁸₆₆. The method of claim ²⁷₆₅, wherein said cleaving produces a polynucleotide having a 3'-hydroxyl group at its 3'terminus.

²⁹₆₇. The method of claim ²⁶₆₄, wherein said removable blocking moiety linked to said solid support is selected from the group consisting of esters, ethers, carbonitriles, phosphates, carbonates, carbamates, borates, nitrates, sugars, phosphoramidate, phosphoramidates, phenylsulfenates, sulfates, sulfones and amino acids, wherein said removable blocking moiety is linked to the 3' position of said nucleoside 5'-triphosphate and is also linked to said solid support.

D¹
con't